Effects of different loading rates and types of biochar on passivations of Cu and Zn via swine manure composting

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Abstract: Pollution of arable land caused by heavy metals in livestock and poultry manure has become a potential threaten to human health in China. Safe disposal of the contained toxic pollution with animal manure by co-composting with biochar is one of the alternative methods. Biochars from different sources (wheat straw, peanut shells and rice husks) amended with different loading rates were investigated for passivations of copper and zinc (Cu and Zn) in swine manure composting. Results showed that the passivation effects of the three types of biochar on Cu and Zn were enhanced with increasing biochar dose. Contents of Cu and Zn measured by diethylenetriaminepentaacetic acid (DTPA) and Community Bureau of Reference (CBR) showed that wheat straw biochar with the loading rates of 10%–13% (w/w) was superior to the other two types of biochar in this study. Compared with the control, sample from wheat straw biochar was more favorable for the bacterial growth of Proteobacteria, Firmicutes and Actinobacteria. In addition, pot experiment showed that organic fertilizer amended with wheat straw biochar could significantly improve the growth of Chinese pakchoi and enzyme activities (superoxide dismutase, peroxidase, polyphenol oxidase and catalase) as compared with the control. Cu and Zn contents of Chinese pakchoi in the organic fertilizer group containing wheat straw biochar reduced by 73.2% and 45.2%, 65.8% and 33.6%, respectively, compared with the group without loading biochar. There was no significant difference in the contents of vitamin C and reducing sugar between the groups of organic fertilizer amended with/without wheat straw biochar, however, there was significant difference compared with the heavy metal addition group. The application of organic fertilizer formed by adding biochar can effectively reduce the adverse effects of heavy metals on crops.

Keywords: biochar; composting material; heavy metal passivation; dosage; swine manure

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1 Introduction

Metal elements of copper (Cu) and zinc (Zn) as feed additives can effectively inhibit the growth of harmful microorganisms in pig intestine and reduce the morbidity and mortality of pigs (Li et al., 2019; Shen et al., 2020). More than 15×10^4 tons of trace element as feed additives were used in breeding livestock and poultry every year in China, and about 55% to 66% of them are not absorbed by animals (Yan et al., 2018; Zeng et al., 2018). Yan et al. (2018) reported that the utilization rates of Cu and Zn were less than 5%, and more than 95% of them entered the environment with feces. Heavy metals cannot be directly removed by microorganisms, or eliminated by physical and chemical methods. Therefore, accumulation of these heavy metals has caused deterioration of soil and water biota, and environmental ecosystem. In addition, these

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contaminants are not only toxic to soil microorganisms, but also negatively affect crop production and quality, which could pose a potential threat to human and animal health (Kumar et al., 2019). As a large pig farming country, China has more than 50% of the world's total farming production, resulting in the accumulation of an estimated 618×10^6 tons of manure each year (Chen et al., 2020a). Considering the pollution of heavy metals in the manure of farming animals, effective approaches to diminish the pollution are necessary and have been widely investigated in China.

Composting has become one of the main technical methods to safely deal with livestock and poultry breeding waste in China and the world (Cheng et al., 2019; Yao et al., 2020; Zhong et al., 2020). Composting utilizes fermentation process to complex and fix the heavy metals while transforming the physical, chemical and morphological properties of the organic matter in composting material, so that the heavy metals are immobilized and less bioavailable for intake of bio-organisms. Deactivated heavy metals are divided into exchangeable, oxidized, reduced and residual in Community Bureau of Reference (CBR) stepwise extraction method. The bio-availability of heavy metals is gradually reduced in the following order: exchangeable, oxidized, reduced and residual, in which exchangeable heavy metal is the most easily absorbed or migrated form by plants (Pérez-Cid et al., 1999). Studies have confirmed that passivation of heavy metals (Cu and Zn) by co-composting with animal waste can increase the residue content, thereby reducing the toxicity of heavy metals (Tang et al., 2020). Although composting did not noticeably change the total amount of heavy metals, it significantly decreased water solubility of Cu and Zn (Ihnat and Fernandes, 1996). However, composting method as the only way to lessen the pollution of heavy metals in livestock and poultry manure is inefficient in inertization of heavy metals due to some limitations, such as less microbial activities, poor cation exchange capacity or low conductivity during composting process. The concentration effect in composting may also result in high level of heavy metal residues in the final composting product.

Previous study reported that biochar has the following characteristics: rich carbon content, large specific surface area, low bulk density, strong adsorption capacity and high stability. Meanwhile, it is a porous carbonaceous material (Zhang et al., 2020). In addition, biochar consists of recalcitrant organic carbon made of aromatic rings that are stacked irregularly, and contains functional groups and active compounds, such as olefin (Leng et al., 2020). Therefore, biochar can provide a fundamental role in passivation of heavy metals during composting process. Schmidt et al. (2014) reported that composting with biochar is an effective way to passivate Cu and Zn for reduction of heavy metals pollution in soil. Another research showed that biochar application in the chicken manure composting significantly decreased the bioavailability of Cu by 90.3% and Zn by 11.7% (Hao et al., 2019). The inoculation of biochar in composting has played a positive role in the passivation of heavy metals, which is mainly due to the special porous structure, ion exchange capability and compound-functional groups of biochar. In the past few years, researchers have devoted their energy to investigate the utilization of biochar as an amendment to stabilize various types of heavy metals (Plácido et al., 2019; Shan et al., 2020; Xu et al., 2020), with focuses on dynamics, enzyme activity and microbial diversity (Lucchini et al., 2014; Huang et al., 2017; Tang et al., 2020). There are few reports, however, on systematic studies of the effects of biochar type and loading rate on the passivation of heavy metals. Since the type and dosage of biochar added could directly determine the physical-chemical properties of composting material and therefore change the overall profile of composting process, more information on the effects of these two parameters are required to better understand the passivation.

This study mainly focused on investigating the effects of different types of biochar at various dosages on levels of Cu and Zn residues and their passivations via swine manure composting. The main objectives were to evaluate the effects of three types of biochars (wheat straw biochar, rice husk biochar and peanut shell biochar) and different loading rates on passivations of Cu and Zn. To evaluate the effect of the biochar-amended composting on plant growth, we performed a pot study using Chinese pakchoi. Upon identifying the best-performing biochar composting, we evaluated the microbial diversity and its effects on the growth, physical-chemical and biological properties of Chinese pakchoi.

2 Materials and methods

2.1 Composting materials

Composting materials in this study consisted of corn straw (CS), swine manure (SM), wheat straw biochar (WS), rice husk biochar (RH) and peanut shell biochar (PS). The SM was provided by Wan Xinyuan Pig Breeding Co., Ltd., China. RH was purchased from Liaoning Jin He Fu Co., Ltd., China. WS and PS were purchased from Henan Sanli Co., Ltd., China. WS, RH and PS were grounded until they could pass through a 2-mm sieve and then prepared by heating air-dried rice straw at 500.0°C for 3 h. The general properties of each raw material are presented in Table 1.

Table 1 Characteristics of composting materials

Material	Moisture (%)	pH	OM (%)	C/N	Cu (mg/kg)	Zn (mg/kg)
SM	75.34±0.61	7.63±0.45	16.70±0.32	14.42±0.15	654.20±0.27	821.40±0.52
CS	7.02±0.53	7.81±0.23	65.20±0.47	51.74±0.51	6.50±0.17	63.30±0.28

Note: SM, swine manure; CS, corn straw; OM, organic matter; C/N, the ratio of carbon to nitrogen. Mean±SD.

2.2 Design of composting

As shown in Table 2, RH, WS and PS were loaded into composting piles at the percentages of 0%, 2%, 5%, 7%, 10% and 13% (w/w), thus the three types of treatments were respectively named as RH1–RH5, WS1–WS5 and PS1–PS5. SM and CS (0.5–1.0 cm) were evenly mixed and loosely loaded into polyethylene foam bins (38 cm×25 cm×41 cm). The weight of loading mixture was about 50 kg. The moisture content of composting material was adjusted to approximately 65% and the ratio of carbon to nitrogen (C/N) was adjusted to 25:1. The experiment was conducted for 35 d and each treatment was performed with three replications. The mixtures were well turned every 3 d during the first two weeks and then left undisturbed to maintain good ventilation and enough oxygen for the rest period of composting. The collection points of temperature were evenly distributed in five locations of composting reactor. A polyethylene fiber rod with three thermocouples (TESTP-K01, Shanghai Hongyi Instrument Co., Ltd., China) was installed in each collection position. A total of fifteen thermocouples were located at three different depths (10, 20 and 30 cm from the top of the foam bin) of composting reactor. The temperatures of ambient and location in composting box were recorded twice per day (09:00 and 17:00 LST) by a digital thermometer receiver (TES-1310, Shanghai Hongyi Instrument Co., Ltd., China). The average value of temperature from thermocouple in the foam bins was calculated and used as the final temperature of composting.

Table 2 Scheme of composting experiment

Treatment	Percentage of biochar in composting (%)					
SM+CS+RH	0	2	5	7	10	13
SM+CS+WS	0	2	5	7	10	13
SM+CS+PS	0	2	5	7	10	13

Note: SM, swine manure; CS, corn straw; RH, rice husk; WS, wheat straw; PS, peanut shell.

Samples were collected from four corners and center layer of the box at the depth of about 15 cm below the top interface of composting piles. Then the five samples were evenly mixed into one sample with a total weight of about 250 g. Samples were collected on 0, 2, 4, 7, 10, 12, 15, 19, 22, 26, 31 and 34 d of the experiment. Each collected sample was divided into two parts: one part was immediately tested for the physical and chemical parameters, the other part was stored as fresh sample at –20.0°C for further analysis (Yu et al., 2018).

2.3 Heavy metals extraction

Total amount of Cu and Zn in each sample was determined by atomic absorption spectrometry (AA6100, Shanghai Tianmei Analytical Instrument Co., Ltd., China). All samples were turned to

ash, boiled and then filtered. The procedure used is briefly described. A total of 0.5 g of each sample was dried in an oven at 105.0°C for 2 h and finely ground until passing through a 60-μm mesh sieve. Sieved samples were placed in a muffle furnace at 500.0°C for 2 h with 0.25 g being removed and accurately weighed in a polytetrafluoroethylene crucible. A total of 5 mL of perchloric acid and 5 mL of nitric acid were added to the polytetrafluoroethylene crucible, then was heated to boil for 20 min on a hot plate at 200.0°C. After returning to room temperature, 5 mL of hydrofluoric acid was added, and continuous heat was applied until samples were nearly dried. This process was repeated until the solution was clear and translucent, eventually heating to near dryness. Samples were dissolved by adding 1 mL of nitric acid. After cooling, it was transferred to a 50-mL volumetric flask and filtered through a 0.45-micron glass fiber filter. The content of heavy metals was measured by diethylenetriaminepentaacetic acid (DTPA). The DTPA extraction can accurately measure the bioactive state of heavy metals with reliable reproducibility. The modified CBR stepwise extraction method was used to analyze the distribution of different fractions in deactivated heavy metals. The details of extraction approaches were previously reported by Xiao (2016).

2.4 Diversity of microbial community

Effect of biochar on the diversity of microbial communities in composting was investigated. The samples for determining microbial diversity were selected from the biochar group with the best passivation effect on heavy metals. Furthermore, the samples for determining microbial diversity were collected from three groups: (1) the control (group C), sampled at the initial of composting; (2) group N, sampled at the end of composting without biochar; and (3) group W, sampled at the end of composting with WS biochar.

We extracted DNA from samples using a DNA extraction kit (Sangon Biotech Shanghai Co., Ltd., China), and determined DNA integrity by electrophoresis on a 1% agarose gel. We quantified DNA using the Qubit2.0 DNA kit (Sangon Biotech Shanghai Co., Ltd., China) according to the manufacturer's instructions. The diversity of microbial community was analyzed by using high-throughput illumina sequencing (MiSeq2500, BioMarker Technologies Co., Ltd., China). After pyrosequencing, we processed sequences with Prinseq software (Prinseq-lite-0.19.5) to remove the low-quality data and improve the syncretic rates of subsequent sequences. We fused double-ended sequences using FLASH v.1.2.7. The sequences with similarities greater than, or equal to 0.97 were grouped into operational taxonomic units (OTUs) using UCLUST v.1.2.22. The ITS sequencing data were classified by using the amount of OTUs. We compared the relative abundance of OTUs throughout the samples to investigate the microbial community based on different treatments.

2.5 Pot experiment of Chinese pakchoi

Pot experiment of Chinese pakchoi (*Brassica campestris* L. ssp. *chinensis* Makino) was conducted to evaluate the effects of heavy metal passivation in the biochar-amended composting on the physical-chemical properties of the plant. Table 3 shows the experimental design with five different treatments (CK, control; HM, heavy metal; CF, chemical fertilizer; OF, organic fertilizer; and WOF, wheat straw biochar and organic fertilizer). The fertilizer or nutrients were added at 2% (w/w) to each pot (20 cm×20 cm×15 cm), which contained a total of 1200 g of soil for each treatment. The ratio of Cu to Zn in the heavy metal group (HM) was 1:1 (w/w), which accounted for 2% of the total soil weight. Five replicates were conducted in each treatment. The pot experiments were performed in a greenhouse with controlled temperatures and moisture (23.0°C (±2.0)°C, 60% relative humidity, 10–12 h light) for four weeks. Enzyme activities including superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) were detected using the respective kits (series No. A003798 for PPO, A002559 for POD, A001896 for CAT, A003540 for SOD, respectively). The contents of vitamin C (Vc) and soluble reducing sugar were also measured during pot experiments using specific kits (series No. A500830 for Vc and BC0235 for soluble reducing sugar, respectively). The enzymes and Vc kits were purchased from Sangon Biotech, Shanghai, China, and the soluble reducing sugar kit was purchased from Solarbio Life Sciences Co., Ltd., Beijing, China.

Cu and Zn contents of Chinese pakchoi were measured as following methods: Chinese pakchoi was picked on 28 d after planting, washed and dried in a 105.0°C oven to a constant weight. We weighed 0.1 g of the ground sample into a 50-mL polytetrafluoroethylene digestion tank, added 3 mL concentrated nitric acid and 2 mL perchloric acid, and heated on a 200.0°C graphite heating plate (DB-1EFS, Shanghai lichen Bangxi Instrument Technology Co., Ltd., China) until completely dissolved, then transferred the solution into a 50-mL colorimetric tube. After filtering, we considered the liquid in the polyethylene plastic bottle as the test sample. The contents of Cu and Zn in the liquid were determined by atomic absorption spectrometer (AA6100, Shanghai Tianmei Analytical Instrument Co., Ltd., China).

Seeds of Chinese pakchoi were purchased from Flower Market of Dalian Xijiao Gardens, China. The soil had the following properties: pH 6.5, organic matter 368 g/kg, available N 79 mg/kg, available P 129 mg/kg and available K 40 mg/kg.

2.6 Analytical methods

pH was determined by using a pH meter with fresh 12 samples suspended in purified water at 1:10 (w/w) (Zeng et al., 2010). We detected electric conductivity (EC) by using an instrument based on fresh samples suspended in sterile water at 1:10 (w/w) (Chikae et al., 2006). Then the suspension was oscillated for 20 min followed by filtration, measurement and data recording. Moisture content was measured after drying composting samples at 105.0°C for 24 h (Zeng et al., 2011). Total organic carbon (TOC) was determined by potassium dichromate and sulphuric acid method. Total nitrogen (TN) content was measured using the Kjeldahl method (Nelson and Sommers, 1980) followed by calculation of the C/N ratio. Cucumber seeds were used for the calculation of germination index (GI) (Zucconi et al., 1981). GI was measured by the method described by Hu et al. (2015).

$$\text{GI (\%)}=(\text{seed germination in treatment (\%)}\times\text{root length in treatment})/(\text{seed germination in control (\%)}\times\text{root length in control})\times100\%. \quad (1)$$

2.7 Statistical analysis

Statistical analysis was performed by using SPSS (IBM SPSS 22.0, Chicago, USA). Duncan's multiple range test was used to compare the treatment effect at the 0.05 significance level. All figures were developed using the GraphPad Prism 7.0 (GraphPad Software Inc., San Diego, CA, USA).

3 Results

3.1 Physical-chemical characteristics of composting

3.1.1 Temperature and moisture

Temperature increased over 50.0°C after 2 d of composting among three types of biochar (Fig. 1). All treatments entered the thermophilic phase (>55.0°C) after 3 d and lasted for about 10 d. The highest temperature of composting piles was 69.5°C in RH4 (4 d), 71.1°C in WS5 (3 d), and 67.1°C in PS4 (4 d). Moreover, there was no significant difference in the number of days of the trial group where the highest temperature occurred, which were 10, 12 and 10 d, respectively in RH4, WS5 and PS4. The change trends of temperature in three types of biochar were very similar during composting process. The temperatures of all the biochar-amended composting were close to the ambient temperature after 35 d. The moisture contents of all trial groups decreased from 65.0% to 20.0%, where the lowest moisture content was 20.3% in RH1, 18.2% in WS4 and 16.6% in PS4.

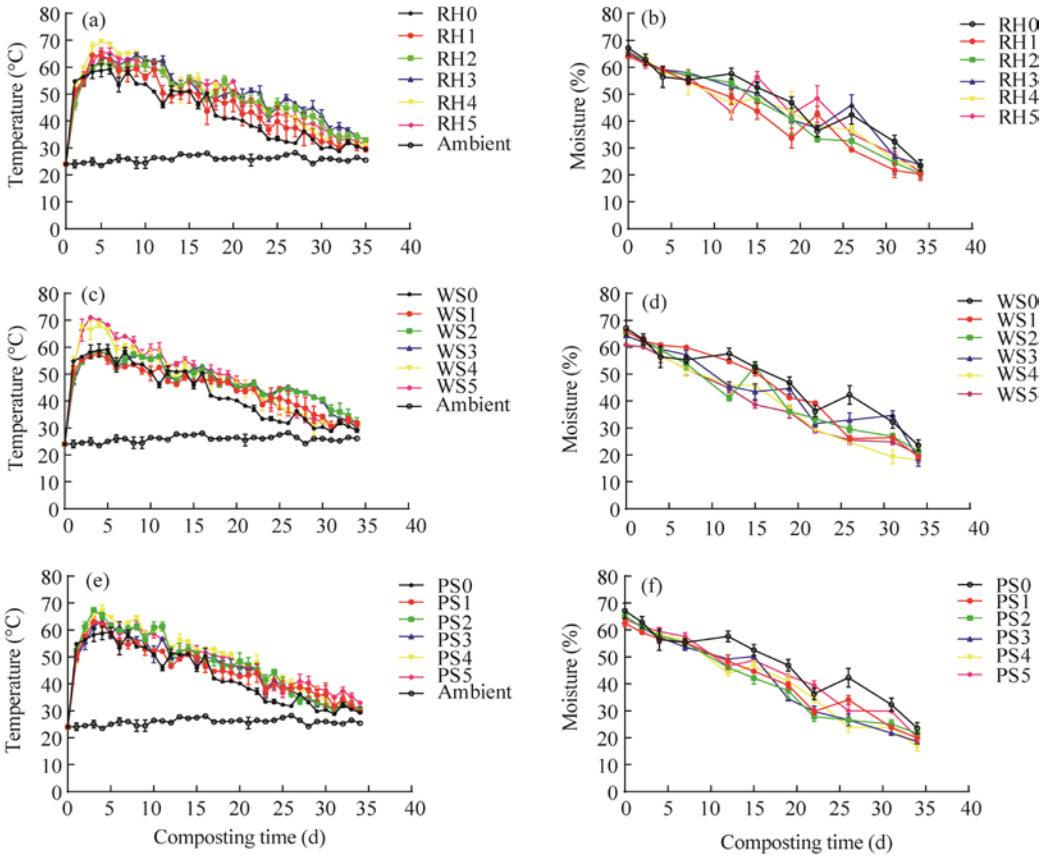

Fig. 1 Changes of temperature and moisture during composting process. RH, rice husk; WS, wheat straw; PS, peanut shell. 0, 1, 2, 3, 4 and 5 represents biochar loading rates of 0%, 2%, 5%, 7%, 10% and 13%, respectively. Bars are standard errors.

3.1.2 pH, EC, TN and C/N

The pH value showed a similar fluctuation trend: firstly increasing, then decreasing, following by increasing and decreasing. At the end of composting, pH value was about 7.6–8.0. EC values in three treatments showed a similar trend with pH values. Moreover, EC values were higher at the end of composting than that of the initial. However, the comparison between the test groups with different dosages of each biochar showed no significant difference (data not shown).

Although the addition amounts of three types of biochar were different, the change trend of TN was similar. No significant difference was found in TN values between control group and experimental group at the end of composting. C/N ratio sharply increased during the first 3 d of composting in all trial groups. Changes of C/N ratio were similar in three types of biochar. Non-significant difference in C/N ratio was observed among different loading rates of each biochar at the end of composting (data not shown).

3.1.3 Germination index (GI)

Compared with no biochar groups (WS0, RH0 and PS0), GI value increased with the loading rate of biochar (Fig. 2). Although there was no significant difference in the loading rates of 7% and 10% in WS, RH and PS, GI value of WS was significant lower than those in RH and PS with 13% loading rate of biochar. The peak value of GI was 96.5% in WS with 10% loading rate, 97.5% in RH with 7% loading rate and 98.5% in PS with 10% loading rate. The significant difference was observed between WS and the other two types of biochar with 5% loading rate (Fig. 2).

3.2 Heavy metals

3.2.1 Cu and Zn proportions extracted by DTPA

Cu (DTPA-Cu) and Zn (DTPA-Zn) proportions extracted by DTPA decreased with increase in

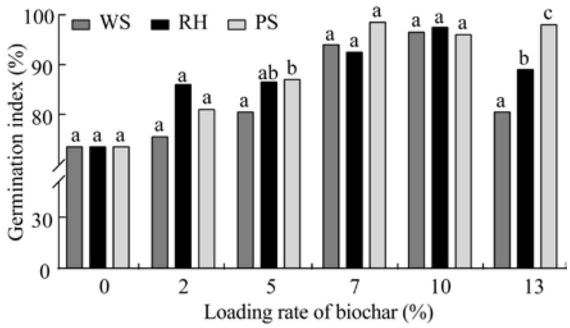

Fig. 2 Germination indices under different types and loading rates of biochar. WS, wheat straw; RH, rice husk; PS, peanut shell. Different lowercase letters indicate significances under different types of biochar with the same loading rate at P<0.05 level.

biochar addition (Fig. 3). WS showed a significantly decreasing amount of DTPA-Cu and DTPA-Zn when loading rate was 5% compared with those of PS and RH. Although the values of DTPA-Cu between RH (18.5%) and PS (19.5%) had no significant difference (Fig. 3a) with 13% biochar loading rate, the value of DTPA-Cu in WS (12.6%) was significant lower than those of the other two types of biochar. DTPA-Zn in WS and PS was 4.5% (Fig. 3b), which was significant lower than that in RH. Compared with the initial values, the final values of DTPA-Cu decreased by 45.3%, 15.2% and 19.5% in WS, PS and RH, respectively. DTPA-Zn decreased by 30.3%, 29.7% and 20.7% in WS, PS and RH, respectively. DTPA-Cu and DTPA-Zn had the highest decline in WS. Furthermore, the difference in WS biochar loading rates between 10% and 13% was not significant.

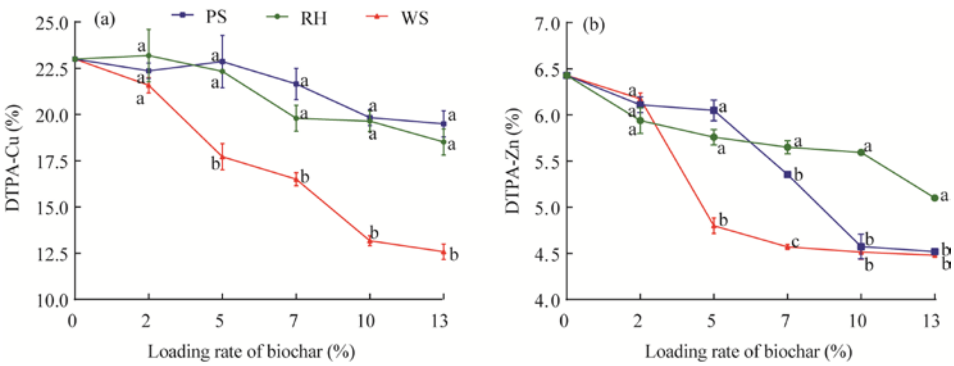

Fig. 3 Percentages of Cu (DTPA-Cu, a) and Zn (DTPA-Zn, b) extracted by DTPA (diethylenetriaminepentaacetic acid) in different loading rates of biochar. PS, peanut shell; RH, rice husk; WS, wheat straw. Different lowercase letters indicate significant differences among different loading rates of biochar at P<0.05 level. Bars are standard errors.

3.2.2 Cu and Zn proportions extracted by CBR

The exchangeable fractions of Cu and Zn decreased, while the residual fractions increased with increasing loading rates in WS (Fig. 4a and b). Compared with the control group (WS0), the residual fraction in WS for Zn increased from 27.5% to 44.3%, and for Cu, it increased from 17.6% to 44.3% with increased biochar loading rates from 0% to 13%. The highest value of residual fractions of Zn and Cu were 27.9% and 39.7% in PS4 (Fig. 4c and d), and 30.5% and 24.9% in RH4 (Fig. 4e and f), respectively. The exchangeable fractions of Zn and Cu in WS5 were significant lower than those in other two types of biochar.

3.3 Microbial community

Based on the results of DTPA and CBR on the extractable Cu and Zn, WS was superior to the other two types of biochar (PS and RH) for the passivations of Cu and Zn. The high throughput

sequencing method was adopted to investigate the microbial diversity of composting in WS. Samples were collected from three groups: (a) group C, sampled at the initial of composting; (b) group N, sampled at the end of composting without biochar; (c) group W, sampled at the end of composting with WS biochar. Among the bacterial phyla (Fig. 5a), Proteobacteria, Firmicutes and Actinobacteria together accounted for about 90% in group W and 70% in group N. The abundance of *Bacillus* (Fig. 5b) in group W was significantly higher than that in group N. Data from ternary diagram (Fig. 6) showed the association and relative abundance among three groups, in which Firmicutes was the main bacteria found in group W and significantly higher than that in group N.

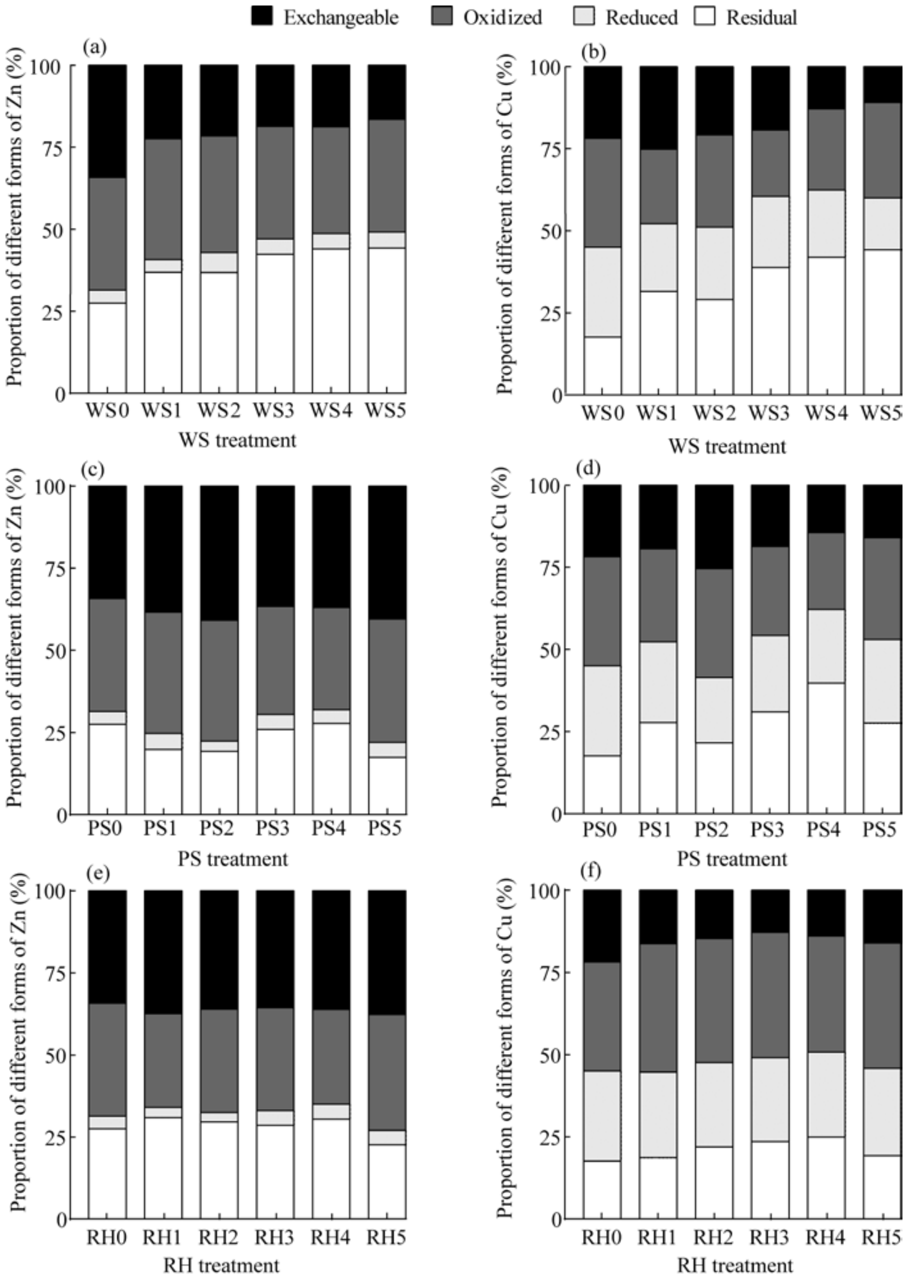

Fig. 4 Proportions of exchangeable, oxidized, reduced and residual forms of Zn and Cu in WS (a and b), PS (c and d), and RH (e and f) treatments by Community Bureau of Reference stepwise extraction method. WS, PS, and RH represent biochars of wheat straw, peanut shell and rice husk, respectively. 0, 1, 2, 3, 4 and 5 represents biochar loading rates of 0%, 2%, 5%, 7%, 10% and 13%, respectively.

3.4 Effects of biochar on growth, heavy metal content and enzyme activity of Chinese pakchoi

Results showed that organic fertilizer in WS was higher than other experimental groups on supporting the growth of Chinese pakchoi as suggested from four indices (height, root length, fresh weight and dry weight) (Fig. 7a–d). The contents of Cu and Zn of Chinese pakchoi (Fig. 7e and f) in WOF were significantly lower than those in HM and OF. Compared with control group and no straw biochar addition group, Cu contents of Chinese pakchoi in WOF reduced by 73.2% and 45.2%, respectively. Zn contents decreased by 65.8% and 33.6%, respectively.

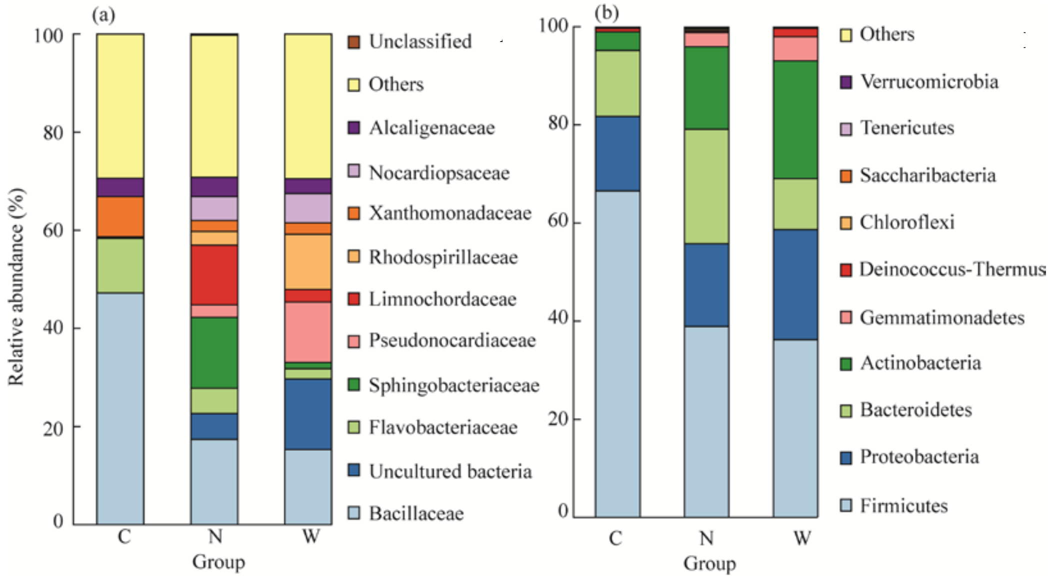

Fig. 5 Bacterial community structure at the phylum (a) and family (b) levels in three experimental groups. The data contained top 10 of the total reads. Group C, sampled at the initial of composting; Group N, sampled at the end of composting without biochar; Group W, sampled at the end of composting with WS biochar.

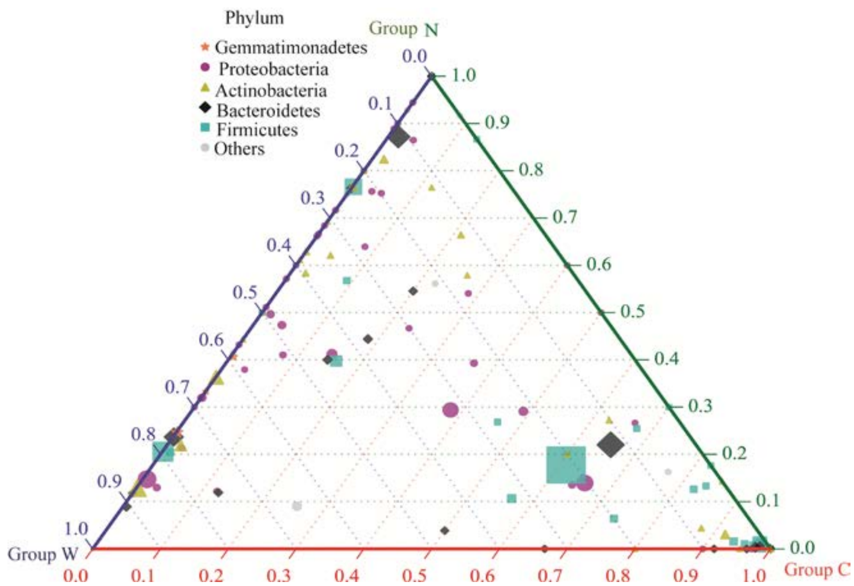

Fig. 6 Relative aboundace of bacterial community illustrated by ternary diagram. Group C, sampled at the initial of composting; Group N, sampled at the end of composting without biochar; Group W, sampled at the end of composting with WS biochar.

Results of biochemical indicators showed that HM had the highest value of SOD enzyme activity among five experimental groups (Fig. 8). However, there were no significant differences in SOD enzyme activity between HM and the rest groups (CF, OF and WOF). According to the results of the other three enzymes, POD, PPO and CAT had the highest activities in WOF compared with other groups (Fig. 8).

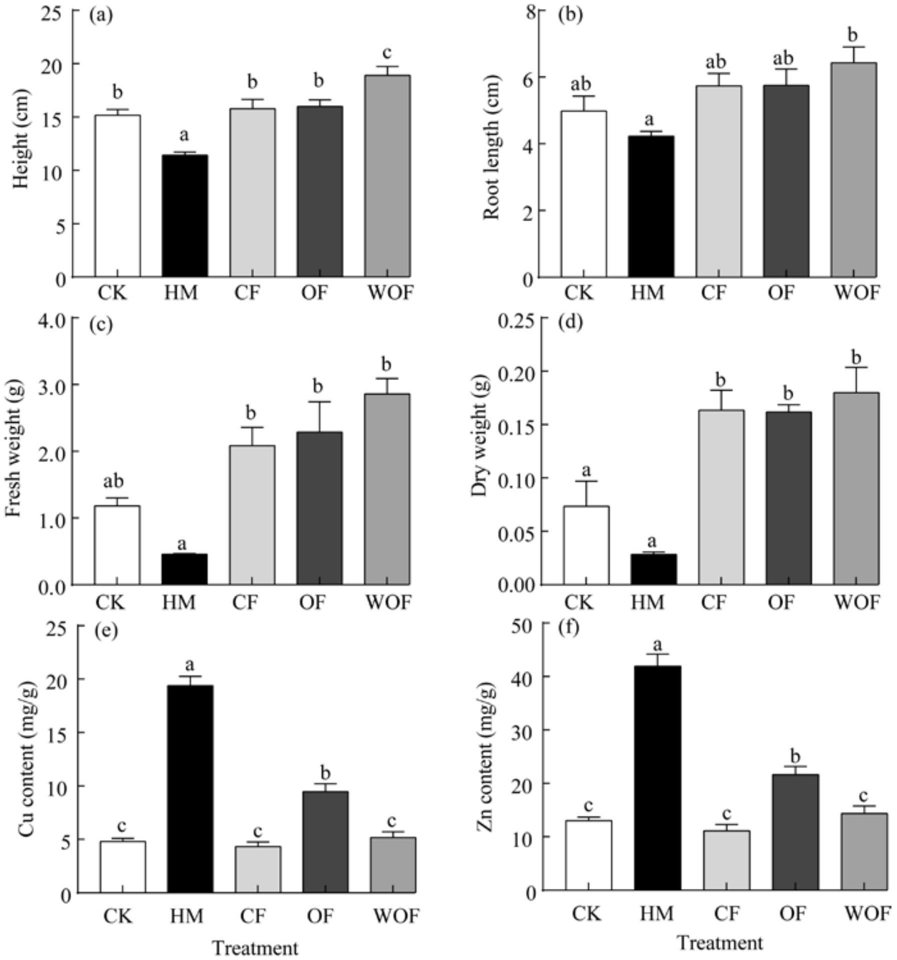

Fig. 7 Growth indices and heavy metal contents of Chinese pakchoi. (a), plant height; (b), root length; (d), fresh weight; (d), dry weight; (e), Cu content; (f), Zn content. Different lowercase letters indicate significant differences among different treatments at *P*<0.05 level. CK, control; HM, heavy metal; CF, chemical fertilizer; OF, organic fertilizer; WOF, wheat straw biochar and organic fertilizer. Bars are standard errors.

4 Discussion

4.1 Physical-chemical characteristics of composting

Generally, one of the most important indices to evaluate the maturity and stability of composting is temperature (Chen et al., 2020b). There was a similar trend with previous studies using biochar as bulking agent (Sánchez-García et al., 2015), i.e., temperatures of the experimental groups with loading biochar were higher than those of control groups (RH0, WS0 and PS0), indicating that biochar as a supplemental material had a positive effect during composting process (Agegnehu et al., 2017; Chen et al., 2017). Besides, temperature of three types of biochar showed a trend of increase with the increase of dosage. Due to the continuous high temperature and metabolism of microorganisms, the moisture of composting gradually decreased.

The final pH was a little higher than 7.0 in all the amended-biochar composting. It might be mainly due to the two factors: (1) the pore structure of biochar that could maintain alkaline

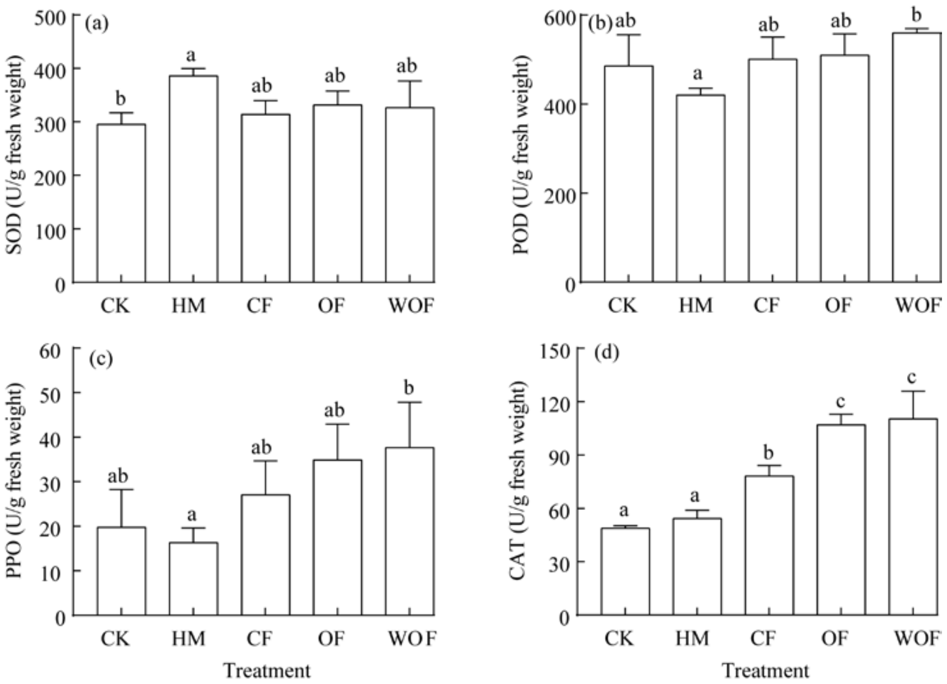

Fig. 8 Enzyme activities of superoxide dismutase (SOD, a), peroxidase (POD, b), polyphenol oxidase (PPO, c), and catalase (CAT, d) under different treatments. CK, control; HM, heavy metal; CF, chemical fertilizer; OF, organic fertilizer; WOF, wheat straw biochar and organic fertilizer. Different lowercase letters indicate significances among different treatments at P<0.05 level. Bars are standard errors.

ammonia; and (2) biochar kept an alkaline state under natural conditions (Sun et al., 2019). The rate of fractionation of ionic molecules and organic matter decomposition directly affected the value of EC during composting process. When the value of EC was below 4.0 mS/cm, the end product of composting was stable and non-toxic to plants (García et al., 1991). However, the value of EC was higher than 4.0 mS/cm at the end of composting in this experiment. It could be due to the active groups and compounds in biochar, such as hydroxyl and olefin. Moreover, a higher dose of biochar as a compost modifier can effectively buffer the volatile fatty acids and increase the mineralization of organic matter in the process of swine manure composting.

TN content fluctuated at the initial of composting and subsequently increased at the end of composting, which was consist with previous study (Wei et al., 2016). Wang et al. (2013) claimed that the volatilization of ammonia nitrogen and decomposition of organic nitrogen compounds were the main reasons for the decreasing TN content in the initial of composting. The upward trend of TN content may be due to the porous structure of biochar that provides more cavities and space for nitrifying bacteria to retain more nitrogen in composting (Iqbal et al., 2015). C/N ratio is the criteria traditionally used to assess the maturity of composting. C/N ratio in all experimental groups was less than 20 at the end of composting. The result was consistent with a previous report (Md Khudzari et al., 2016). C and N can be used as sources of energy and nutrients by microorganisms for their growth and metabolism (Yang et al., 2019). Therefore, C/N ratio would change as the C and N sources are utilized during composting process.

GI is usually used as the evaluation of the phytotoxic substances contained in composting, and is considered as one of the most sensitive parameters to determine the maturity of composting (Zucconi et al., 1981; Tiquia, 2010). Results of GI confirmed that phytotoxicity in the end product of composting decreased because GI value exceeded 80% in the experimental groups, indicating that the loading of biochar in composting effectively reduced the potential toxicity of heavy metals.

4.2 Comparison of DTPA and CBR methods

DTPA extractable fraction in heavy metals represents the metallic elements that can be absorbed

by plants: the smaller its proportion in the total content, the lower in bio-availability of heavy metals and the better in passivation effect of biochar (Jing et al., 2020; Surgutskaia et al., 2020). The immobilization of heavy metals by biochar may be attributed to the following reasons: (1) charge effect: the evolution of pH value will change the ability of biochar to carry charge. With the loading rate raised up in composting, the electrostatic attraction between heavy metals and negative charge is enhanced (Sun et al., 2019); (2) compound-functional group: a large amount of functional groups cover over the biochar surface, such as carboxylic, hydroxyl and alcohol groups. Therefore, these compound-functional groups and heavy metals are more likely to form inert composite aggregates (Tang et al., 2013); and (3) microbial activity: biochar can provide a larger specific surface area for microbial survival and metabolism, in turn, the metabolites of these microorganisms act on the compound-functional groups of biochar, thus promoting the passivation of heavy metals in the swine manure (Chen et al., 2017). There was a significant difference in the contents of Cu and Zn between WS and the other two types of biochar. WS may has more porosity, larger specific surface area and more ability of surface functional groups to carry charge, which can lead to stronger adsorption ability of metal after transformation, compared with other two types of biochar.

The bio-availability and toxicity of heavy metals in the environment mainly depend on their chemical speciation, which can be evaluated using the CBR extraction method (Guo et al., 2020). The metals in the material can be categorized into four fractions: exchangeable, oxidation, reductive and residual fraction (Keshavarzifard et al., 2019). In this study, the residual fraction increased with the increasing dosage of WS, indicating high-dose addition group had a more positive effect on the passivation of heavy metals than the low-dose one. The porous structure of biochar had a positive impact on heavy metal adsorption due to their affinity to retain heavy metals, thus preventing heavy metals from leaching out. Feng et al. (2020) confirmed that well-developed pore structure might promote electron transfer between biochar and chemical compound. Furthermore, oxygen-containing functional groups of biochar could adsorb and fix heavy metals due to the complexion and co-precipitation with heavy metal ions.

However, metallic elements will not disappear from the soil, which are merely transformed into various forms, or transferred, dispersed and enriched. The auxiliary materials added in composting, such as biochar, can passivate the heavy metals in livestock and poultry manure, but the carry ability is limited. According to the results of DTPA and CBR methods, the passivation effect of heavy metals was not significantly improved when the loading rates in WS increased from 10% to 13%. On the contrary, high dosage of biochar may cause harm to the environment.

4.3 Microbial community

Zhang et al. (2019) reported that the mineralization and immobilization of nutrients can be influenced by microbial diversity and activity, thus affecting the process of composting. The results of high-throughput sequencing in this study were consistent with the previous reports (Xu et al., 2016; Awasthi et al., 2020), which Proteobacteria, Firmicutes and Actinobacteria, the dominant microorganisms in composting played key roles in the degradation of organic matter. These microorganisms could be protected by the pore structure of biochar, thereby increasing the microbial diversity and biomass (Palansooriya et al., 2019). Additionally, the metabolism of microorganisms may facilitate the formation of compound-functional groups on the surface of biochar, thus enhancing the ability of biochar to form inert complexes with heavy metals (Tang et al., 2013; Chen et al., 2017).

4.4 Pot experiment of Chinese pakchoi

The end products of composting with biochar as additive can improve the nutrient status of soil, enhance the activity of soil rhizosphere microorganism, promote the aeration of soil and increase the enzyme activity of crops (Agegnehu et al., 2017; Guo et al., 2020). In addition, the amended-biochar composting minimalized the toxic of Cu and Zn to the plants. The results of this study were consistent with the previous reports (Khan et al., 2017; Nie et al., 2018). In terms of growth indices and heavy metal contents of Chinese pakchoi, the results of WOF were better than other treatments, indicating that the end products of biochar in composting not only improve the

plants biomass but also play a positive effect on passivation of heavy metal. Yang et al. (2016) confirmed that heavy metals can seriously impact enzyme activity. SOD activity in control group (HM) was higher than that in WOF, which could be mainly due to the enhancement of defensive enzyme activity in Chinese pakchoi with heavy metal stress. However, enzyme activities of PPO, POD and CAT in WOF were significant higher than that in HM, which may be due to the main reason that various beneficial microorganisms were dominant in WOF, including the *Bacillaceae* and *Bacilli*. As the dominant community of rhizosphere microorganisms, they played roles in stimulating the metabolism of Chinese pakchoi (Han et al., 2020). It was coincident with previous study (Montiel-Rozas et al., 2016) that the increase in abundance of rhizosphere microorganisms in composting with biochar was more conducive to the utilization of organic matter in fertilizer. Rayo-Mendez et al. (2019) claimed that organic fertilizer amended with biochar could improve the intrinsic quality of crops due to the beneficial microorganisms promoting the utilization of fertilizers. Meanwhile, the passivation of heavy metals reduced the toxicity of fertilizers to crops, thus promoting the growth.

5 Conclusions

Our results confirmed that heavy metals in poultry and livestock manure can be effectively immobilized by co-composting with biochar. The passivation effect of Cu and Zn is more significant with a higher loading rate of biochar than that of without biochar. Moreover, wheat straw biochar was more efficient in passivations of Cu and Zn than other two types of biochar. Besides, wheat straw biochar could well-balance the relative abundance of bacteria at the phylum and family levels. Organic fertilizer from wheat straw biochar amended composting had the potential to improve the plant rhizosphere microbial communities, facilitate plant growth and enhance fertilizer utilization. For effective passivations of Cu and Zn, we recommended 10%–13% loading rates of wheat straw biochar in composting.

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